(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 July 2002 (18.07.2002)

PCT

(10) International Publication Number
WO 02/056019 A1

(51) International Patent Classification[7]: G01N 33/543, 33/558

(21) International Application Number: PCT/EP02/00437

(22) International Filing Date: 9 January 2002 (09.01.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01200146.7 15 January 2001 (15.01.2001) EP

(71) Applicant *(for all designated States except US)*: **DSM N.V.** [NL/NL]; Het Overloon 1, NL-6411 TE Heerlen (NL).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **KEIZER, Gerrit, Dirk** [NL/NL]; Swingdreef 27, NL-3845 BV Harderwijk (NL).

(74) Agents: **MATULEWICZ, Emil, Rudolf, Antoni** et al.; DSM N.V., DSM Patents & Trademarks, Office Delft (600-0240), P.O. Box 1, NL-2600 MA Delft (NL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/056019 A1

(54) Title: TEST DEVICE

(57) Abstract: The present invention describes a test device which comprises a test strip and a sample receiving means having a predetermined volume. In a preferred test device two more test strips are present which are positioned parallel to each other.

TEST DEVICE

Field of the invention

The present invention relates to a test device for determining the presence, absence or amount of an analyte in a liquid test sample.

Background of the invention

Test strips are used for the detection of analytes in liquid samples such as urine, blood, milk, meat juice, etc. in the diagnostic field.

The analytes to be detected can be hormones, glucose, antibiotics, etc.

In general, the test devices based on strip technology have liquid sample receiving means to which a certain amount (e.g. a fixed number of drops) of liquid has to be added or which is dipped (for a limited time interval or otherwise) into the liquid sample.

For the first mentioned devices care has to be taken to ensure that the correct amount of liquid is added. Moreover, in many cases the test strip is surrounded by a plastic housing which makes the device expensive and, as such a device can not be reused results in the generation of a lot of waste.

For the second group of devices, where the strip is dipped into the liquid, care has to be taken that the liquid level is not too high to avoid wetting too much of the dipstick or that the level of the liquid is not too low, which might result in a too small amount of the sample being absorbed on the test strip.

Summary of the invention

The device according to the invention potentially solves some or all of the problems mentioned above. The amount of sample liquid is less critical and moreover the test device is simple and inexpensive.

The present invention provides a test device for determining the presence, absence or amount of an analyte in a liquid test sample which comprises:

I at least one teststrip comprising at least:

- a sample receiving zone;
- a reaction zone; and,
- an indication zone,

the teststrip comprising a material which makes transport of the analyte from the sample receiving zone, preferably via the reaction zone, to the indication zone, possible and in which, the analyte, if present, is directly or indirectly detectable in the indication zone; and

II a sample receiving means which is in functional connection with the sample receiving zone of the teststrip, so that when the sample is added to the receiving means the liquid sample is in liquid contact with the sample receiving zone and the sample receiving means will receive no more liquid than a predetermined quantity,

wherein when the device is placed on a horizontal plane the teststrip makes an angle of from 10 to 90° with the horizontal plane.

The present invention also provides a kit comprising:

- a teststrip as defined in any one of the preceding claims;
- a sample receiving means of the invention; and
- packaging.

The present invention further provides for the use of a test device or test of the invention to determine the presence, absence or amount of an analyte.

The present invention also provides a method for detecting the presence, absence or amount of an analyte in a liquid test sample, comprising:

- adding the test sample to the sample receiving means of a test-device of the invention;
- identifying, and optionally quantifying, any detectable change in the indication zone of the test-strip of the device.

Brief description of the figures

Fig 1 shows an example of a test strip according to the invention

Fig 2 shows a test device having two test strips

Fig 3 shows the side view of the cross section test device of figure 2

Fig 4 shows a test device having two test strips

Fig 5 shows the side view of the cross section test device of figure 4

Fig 6 shows the side view of the cross section on a test device

Fig 7 shows the side view of the cross section on a test device

Detailed description of the invention

An 'analyte' is the compound or composition to be detected or measured in the liquid test sample. The analyte of interest may be for example a protein, a peptide, an amino acid, a nucleic acid, a hormone, a steroid, a vitamin, a natural or chemical substance, a contaminant, a drug including antibiotics or drugs of abuse, such as heroin, LSD, cocaine, morphine, ecstasy, marijuana. The analyte may be a sugar such as glucose.

In general, the test strip will comprise a transport material which is able to transport the liquid from the sample receiving zone to the indication zone. Advantageously absorbing or chromatographic materials can be used. Examples of materials which may be used are paper, nitrocellulose and porous polymers. Preferably the liquid transport material will be supported by a solid material such as plastic like PVC or PE.

The dimension of the test strip may vary. In general the length will be greater than the width, for example at least three times, preferably five times, more preferably seven times, even more preferably ten times greater than the width. The liquid transport material may be a single or a plurality of materials, as long each of the zones is in liquid contact with the adjacent zone.

The sample-receiving zone (1), receives the liquid test sample and the wetting of the test strip will start. The receiving of the test sample will continue until at least some of the test sample (including the analyte) is transported from the sample- receiving zone to the reaction zone (2) and subsequently to the indication zone (3). In one embodiment of the present invention, filtration means can be present. This filtration means may be a separate material placed before the sample-receiving zone. This filtration means can be used to remove compounds or particles present in the liquid test sample and which might interfere with the liquid transport through the test strip or the binding or reaction in the

reaction or indication zone. Advantageously such a filtration means is mounted on top of the sample-receiving zone of the test strip.

In another embodiment of the present invention after the indication zone, an extra absorbing zone (4) may be advantageously present. This absorbing zone is advantageously applied in case the flow of the analytes or labelled receptor is much lower than the flow of the liquid sample in the test strip and/or in cases where only of very low amounts of analytes to be tested are present in the liquid sample.

In general in the reaction zone the analyte, which enters the receiving zone and which is transported to the indication zone, will be converted (for example labelled) into a compound or form which is directly or indirectly detectable in the indication zone. 'Converted' means that a chemical or physical reaction or coupling may take place. Directly means that analyte, which might be converted, is detected. Indirectly means that active ingredients present on the reaction zone which are intended for the chemical or physical reaction or coupling with the analyte, and which are not reacted or coupled, are detected.

According to one embodiment of the invention in the reaction zone a labelled receptor analyte may be present. The transport zone allows the transport of the analyte and/or the labelled receptor which may or may not be bound to the analyte to the indication zone.

The labelled receptor can be any compound which is capable of binding with the analyte, for example, monoclonal or polyclonal antibody or may be another binding biochemical, microbial or chemical substance or reagent which might bind to the analyte. In cases where an antibody is used either whole antibodies or fragments thereof may be employed. Single chain antibodies, chimeric antibodies, or CDR-grafted antibodies may also be employed. Binding may take place through chemical or physical binding.

In the indication zone the labelled receptor, free of analyte, can be bound on a control zone present in the indication zone. Upon binding, the control zone changes to a signal that can be detected for example a signal visible to the eye. A test zone present in the indication zone may bind the labelled receptor coupled to analyte. Here a less intense colour (compared to the control zone) indicates that the analyte is not present in a sufficient amount (negative result). In case the test zone is equal or more intense than the control zone a high amount of analyte is present (positive result).

According to another embodiment of the invention, in the reaction zone a labelled component is present which can bind to a receptor present in reaction or indication zone. The labelled component competes for binding with the unlabelled analyte in the sample.

The unlabelled receptor can be any compound which is capable of binding with the analyte, for example, monoclonal or polyclonal antibodies, or may be any other biochemical, microbial or chemical structure substance or reagent which might bind to the analyte. The sample may be a fluid such as urine, blood, milk, meat juice or pathological samples. The test sample is generally a liquid. In some embodiments a liquid test sample may be prepared by dissolving a solid or gas into an appropriate liquid.

The above mentioned examples of the use of the test strip to detect an analyte are given to illustrate the present invention. The invention is not limited to these examples. The skilled person will appreciate that other binding and detection mechanisms or means are possible.

'Label' is any substance which is attached to or part of the receptor and which is capable of producing a signal that is detectable by visual or instrumental means. Direct visible labels are preferred. Examples thereof include colloidal metallic (for example gold) and non metallic (for example coloured latex) particles and dye particles. Labels may also be generated enzymatically or an enzyme.

According to a preferred embodiment at least two test strips (6 and 7; 6' and 7') which are positioned parallel to each other are present in the test device (5 and 5'). In this way it is possible to detect in one test device the presence or absence of more than two analytes. Embodiments of the invention include devices where at least three, four, five, seven or at least ten strips are present, preferably in parallel.

Advantageously, the transport material of the test strips, when multiple strips are present, is not in liquid contact with each other, optionally except for the sample receiving zone (8, 8' and 8").

According to one embodiment the transport material of the multiple strips have enough distance from each other to prevent liquid flow from one strip to another, whereas the transport materials are supported on one common support means. Therefore one support means together with several lanes of transport material may form the multiple test strips.

According to another embodiment separate test strips are linked to each other together in such a way that they do not have liquid contact with each other and whereby the indication zones of each strip are still visible. For example the test strips are fixed (for example glued) parallel in a common basis, or the test strips are sealed in parallel position between two foils. To a further preferred embodiment the support means for the test strips and the sample receiving means are dimensioned in such a way that they form together in one part the test holder (figure 2, 3). In this case test holder will be made from one material for example a plastic like polyethylene. The test holder will therefore comprise the support of the test strip and the liquid receiving means and optionally a distance holder.

In a preferred embodiment the distance holder (9") forms together with the sample receiving means (8") one part (figure 6,7). According to this embodiment this one part can be re-used whereas only the at least one test strip is replaced for the next test process.

In one embodiment of the invention the reaction and indication zones are a single zone, typically these embodiments will involve situations where the 'label' is generated or only becomes detectable in the presence of the analyte to be detected.

An important aspect of the invention is the angle of from 10 to 90° of the test strip with the horizontal. Preferably this angle is from 15 to 70°, more preferably from 20 to 60°. According to one embodiment this angle is obtained by using a distance holder (9, 9' and 9") which is placed at the bottom side of the test strip on the opposite side of the sample receiving zone. This distance holder may be of any material. In case the test strip support and holder form one part, the material may be chosen the same. The distance holder may also be a separate means which is connected with the test strip before use.

The liquid receiving means is constructed in such a way that it may contain a predetermined amount of liquid. According to a preferred embodiment of the invention the height (8, 8'and 8") of the liquid receiving means determines the amount of liquid test sample. For example a height of from 1 mm to 50 mm , preferably from 1 mm to 25 mm and even more preferably 1 mm to 10 mm is chosen. Preferably enough liquid samples are added to the liquid receiving means to substantially fill this means. In case more liquid sample would be added, the liquid sample would overflow. In this way it is possible to limit the maximum amount of the liquid test sample in the sample receiving means. Advantageous this height and the position of the reaction zone of the test strip are

chosen in such a way that when the sample receiving zone of the test strip is in liquid contact with the sample receiving means, the maximum liquid level in the sample receiving means is below the reaction zone of the test strip.

The test strips which comprise the liquid transporting material may absorb water from the air, especially under storage conditions in humid surroundings. This absorption might negatively influence the transport behaviour of the test sample in the test strip and might also interfere with the compounds present in the reaction and indication zone.

Therefore, in general desiccants are used to keep the test strip dry during storage. In a preferred embodiment of the present invention the at least one test strip is sealed in foil which prevents the possible absorption of water from the air. This foil is removed before the test strip is used for testing a liquid sample.

The present invention also provides a kit comprising:

- test strip(s) according to the invention;
- sample receiving means according to the invention; and
- packaging.

The test-strip(s) and sample receiving means may be assembled together or be separate in the kit. In the latter case means for assembling the two such as glue may also be provided.

The kit may also comprise one or more of:

- a distance holder according to the invention;
- solutions for diluting the sample if appropriate;
- a positive control comprising a solution containing a known amount of the analyte being tested for;
- a negative control comprising a solution which does not contain the analyte to be tested for;
- instructions for using the test-device of the invention;
- means for detecting the binding of the analyte to the receptor if this is not visually detectable;
- a colour chart for analysing the charge, if any, in the indication zone.

The test-device of the invention may be used to detect disease conditions, susceptibility to disease conditions or drug or alcohol. For example, the devices may be

used to detect glucose in urine or blood to detect diabetes or to monitor the blood sugar level of a diabetic in order that they know when to inject insulin or modify their sugar intake. The test-device of the invention may provide qualitative, quantitative or semi-quantitative results. The indication zone may change colour if the analyte is present and such colour change may be qualitative, semi-qualitative or quantitative. Alternatively, some other property of the indication zone may change in the presence of the analyte. Serial dilutions may be used to help render the device more quantitative.

The change in the properties of the indication zone may be compared to results obtained using standard solutions containing a known concentration of the analyte to be tested for. In some embodiments the colour change, if any, of the indication zone may be measured against a colour chart indicating the expected colour change for known concentrations of analyte. The change in the indication zone may be measured by eye or using a machine. The use of a machine to use the test-device of the invention and analyse the results may increase sample throughout.

The test-device may be used to detect the presence, absence or amount of infectious agents such as viruses and bacteria or of toxins derived from such agents such as tetanus toxin. The devices may also be used to monitor for environmental pollutants.

CLAIMS

1. A test device for determining the presence, absence or amount of an analyte in a liquid test sample which comprises:

   I at least one teststrip comprising at least:
   - a sample receiving zone;
   - a reaction zone; and,
   - an indication zone,

   the teststrip comprising a material which makes transport of the analyte from the sample receiving zone, preferably via the reaction zone, to the indication zone, possible and in which, the analyte, if present, is directly or indirectly detectable in the indication zone; and

   II a sample receiving means which is in functional connection with the sample receiving zone of the teststrip, so that when the liquid sample is added to the receiving means the liquid sample is in liquid contact with the sample receiving zone and the sample receiving means will receive no more liquid than a predetermined quantity,

   wherein when the device is placed on a horizontal plane the teststrip makes an angle of from 10 to 90° with the horizontal plane.

2. A test device according to claim 1, wherein the sample receiving zone is present at the lower part of the teststrip.

3. A test device according to claim 1 or 2, comprising at least two teststrips which are positioned parallel to each other.

4. A test device according to any one of the preceding claims wherein

the reaction zone and detection zone of the teststrip are situated above the level of the liquid sample when the liquid sample is added to the sample receiving means.

5. A test device according to any one of the preceding claims wherein:
- the reaction zone comprises a receptor capable of binding the analyte, if present, the binding of the receptor to the analyte being detectable by direct or indirect means; and/or
- the analyte if present is converted in the reaction zone to a detectable form.

6. A test device according to any one of the preceding claims wherein the reaction zone and indication zone are the same zone.

7. A kit comprising:
- a teststrip as defined in any one of the preceding claims;
- a sample receiving means as defined in any one of the preceding claims; and
- packaging.

8. Use of a test device according to any one of claims 1 to 6 or a kit according to claim 7 to determine the presence, absence or amount of an analyte.

9. A method for detecting the presence, absence or amount of an analyte in a liquid test sample, comprising:
- adding the test sample to the sample receiving means of a test-device according to any one of claims 1 to 6;
- identifying, and optionally quantifying, any detectable change in the indication zone of the test-strip of the device.

10. Use according to claim 8, or a method according to claim 9, wherein the presence or absence of the analyte is indicative of a particular disease state, susceptibility to a particular disease state or of drug or alcohol abuse.

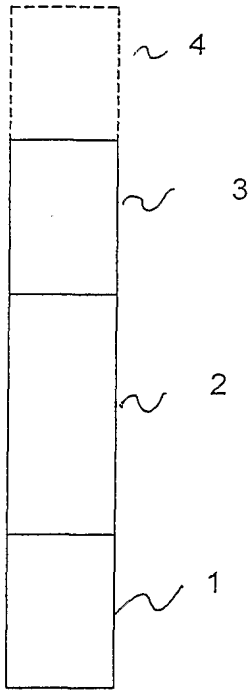

Fig 1

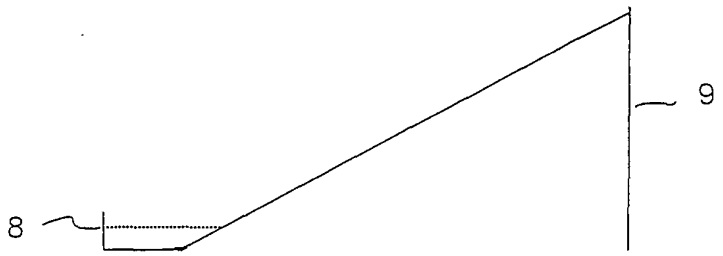

Fig 3

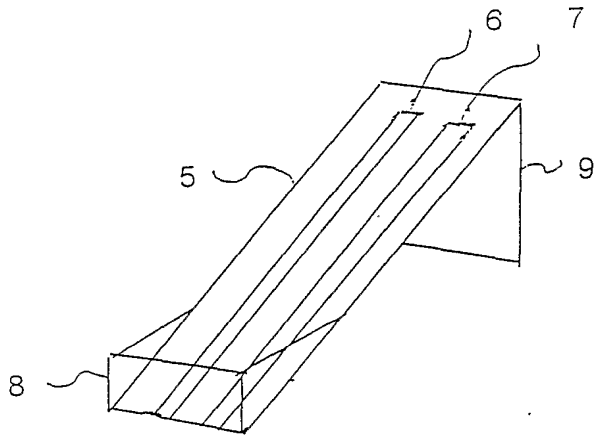

Fig 2

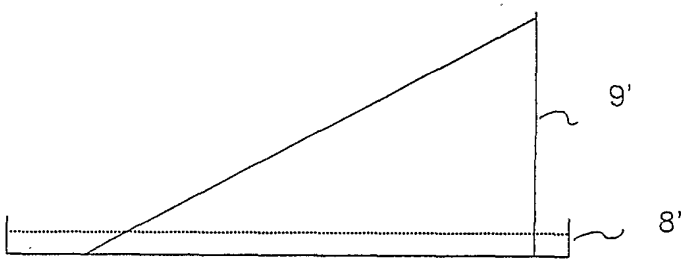

Fig 5

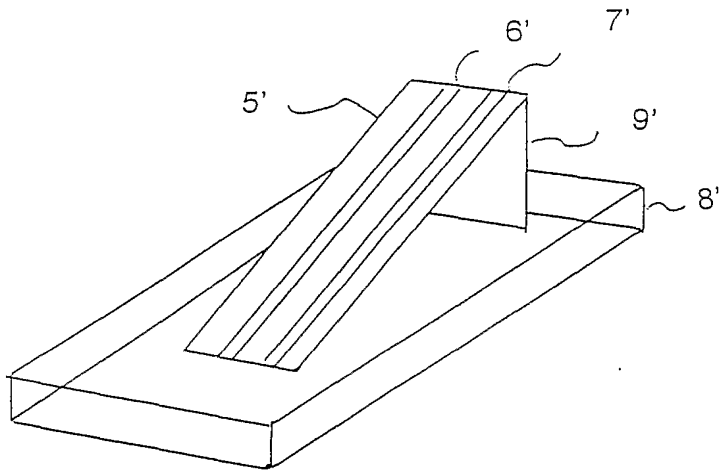

Fig 4

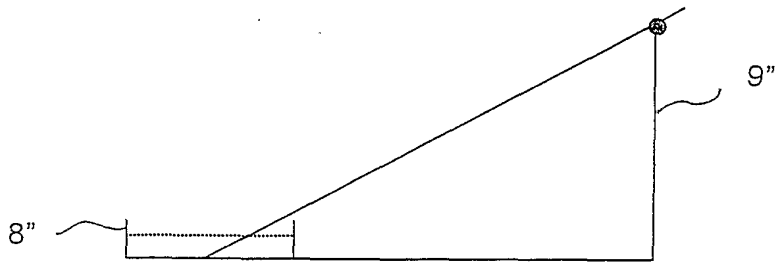

Fig 7

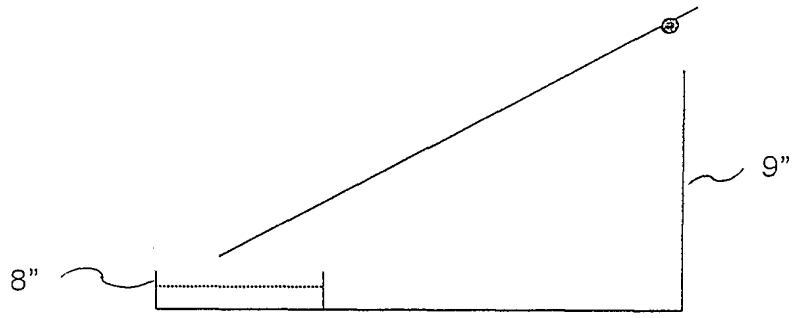

Fig 6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/00437

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/543 G01N33/558

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 987 550 A (SYNTRON BIORESEARCH INC) 22 March 2000 (2000-03-22) column 14, paragraph 4; claims 1,12,13; figures 2,3 column 8, paragraph 4 -column 9, paragraph 1 ---	1-10
A	EP 0 874 240 A (FUJIREBIO KK) 28 October 1998 (1998-10-28) abstract page 5, paragraph 3 ---	1-10
A	EP 0 321 260 A (EASTMAN KODAK CO) 21 June 1989 (1989-06-21) page 2, paragraph 4; claims 1,2 --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 April 2002	07/05/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bigot-Maucher, C

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int[redacted]tional Application No
PCT/EP 02/00437

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 07016 A (AVITAR INC) 10 February 2000 (2000-02-10) abstract	1-10
P,X	US 6 203 757 B1 (CHAN LIANG ET AL) 20 March 2001 (2001-03-20) claim 1	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/00437

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 0987550	A	22-03-2000	AU	2017799 A	10-04-2000
			EP	0987550 A2	22-03-2000
			WO	0017651 A1	30-03-2000
			US	2001021536 A1	13-09-2001
EP 0874240	A	28-10-1998	JP	10300750 A	13-11-1998
			EP	0874240 A1	28-10-1998
			US	6221625 B1	24-04-2001
			US	2001005585 A1	28-06-2001
EP 0321260	A	21-06-1989	US	4988627 A	29-01-1991
			CA	1302249 A1	02-06-1992
			DE	3872499 D1	06-08-1992
			DE	3872499 T2	11-02-1993
			EP	0321260 A1	21-06-1989
			JP	2008745 A	12-01-1990
WO 0007016	A	10-02-2000	US	6194224 B1	27-02-2001
			WO	0007016 A1	10-02-2000
US 6203757	B1	20-03-2001	NONE		